## 77* CFTR and tight junctions in cultured bronchial epithelial cells

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Airway epithelial salt and water transport takes place through paracellular and transcellular pathways. This transport depends critically on the epithelial sodium channel (ENaC) and the cystic fibrosis transmembrane conductance regulator (CFTR) operating in concert with the paracellular pathway and the tight junctions (TJs).
Normal (16HBE14o-), cystic fibrosis (CFBE41o-), and corrected CFBE41o- (CFBE41o-pCep4) airway epithelial cell lines were cultured under isotonic conditions.
Transepithelial electrical resistance (TEER) was measured as indicator of the tightness of the cultures. Morphology was investigated by immunofluorescence and paracellular permeability by lanthanum nitrate or [14C] mannitol as permeability markers.
CFBE41o pCep4 cells developed lower TEER than CFBE41o- cells. Addition of a specific inhibitor of CFTR CFTRinh172) to 16HBE14o- and CFBE pCep4 cells resulted in a timedependent increase in TEER whereas stimulation of CFTR by IBMX and forskolin caused a decrease. Permeability to lanthanum and [14C] mannitol was lower in 16HBE14o- cells exposed to CFTRinh172 and in CFBE41o- cells compared to untreated 16HBE14o- and CFBE41o pCep4 cells, respectively. 16HBE14o- cells exposed to IBMX and forskolin showed higher permeability to lanthanum but lower permeability to [14C] mannitol compared to control. Immunofluorescence revealed a disorganisation of F-actin and α-tubulin in 16HBE14o- exposed to CFTRinh172 and in CFBE41o- cells. Changes in F-actin and α-tubulin in 16HBE14o- cells exposed to IBMX and forskolin were less apparent than for CFTRinh172.
The above results suggest the possibility of an interaction between the activity of CFTR and the TJ protein complex via the cytoskeleton.
Supported by: The Swedish Science Research Council and the Swedish Heart Lung Foundation.

## 78* PDZ protein interaction: the missing link between CFTR and mucins

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Mucins are the main components of the mucus layer covering epithelial surfaces that line luminal organs. They act as lubricants, protectors against pathogens and as an extensive matrix were antimicrobial agents and commensal bacteria reside. Alterations in mucin composition and expression are often correlated to diseases such as inflammation, infections and cancers. The membrane-tethered mucins MUC3, MUC12 and MUC17 are gathered on chromosome 7q22 and expressed in the gastrointestinal tract. They share features common for membrane-associated mucin and more over Class I PDZ motifs on their extreme C-termini, enabling them to act as ligand for PDZ domain-containing proteins.
We report novel interactions between membrane-tethered mucins and PDZ proteins ubiquitously expressed in a wide range of tissues. The human mucin MUC17 is able to bind the PDZ scaffold protein PDZK1 through its C-terminus, an interaction that we prove is crucial for the localization of MUC17 at apical surfaces of enterocytes. Furthermore, we demonstrate that MUC3 plays a part as a novel ligand to the Golgi-associated PDZ and coiled-coil motif containing protein GOPC, known to entrap the C-terminus of the ion channel CFTR in a PDZ-dependent manner. Coexpression of CFTR, GOPC and MUC3 revealed that MUC3 counteracts the increase of CFTR levels due to GOPC overexpression. These reports suggest for the first time a link between ion channels and membrane-associated mucins and cast insight into the roles of mucins in regulatory networks at apical surfaces.

## 79 Regulation of mucin 5AC expression by arachidonic acid metabolites

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Cytosolic phospholipase A2 (cPLA2) is a key enzyme controlling the release of arachidonic acid (AA) from membrane phospholipids. Conversion of AA by cyclooxygenases (COX) and lipoxygenases (LOX) generates prostaglandins and leukotrienes, respectively. We have previously observed that cPLA2 is involved in the mucus overproduction and MUC5AC expression in CF mice. Our aim is to identify AA metabolites and the signalling pathways involved in MUC5AC expression in NCI-H292 bronchial epithelial cell line. Measurements of MUC5AC expression by ELISA and qPCR showed that PMA-induced MUC5AC production was mimicked by AA and inhibited by cPLA2 inhibitors. MUC5AC expression was inhibited by a general LOX inhibitor (NDGA) but not by COX inhibitors (aspirin, NS398). Inhibitors of 12-LOX (CDC, baicalein), but not those of 5-LOX or 15-LOX, reduced MUC5AC expression. These inhibitors did not reduce IL-8 secretion. In agreement, 12-HETE, the first AA metabolite by 12-LOX, stimulated MUC5AC production. Baicalein reduced ERK activation (western blot) and SP-1 translocation (EMSA) induced by PMA. In addition, 12-HETE activated ERK and SP-1 translocation. Both 12-R and 12-S LOX are expressed in NCI-H292. Undergoing experiments using siRNAs will help us to elucidate the contribution of each form of LOX to MUC5AC secretion. In conclusion, our data suggest that 12-lipoxygenase pathway is involved in MUC5AC production via ERK and SP-1-dependent mechanisms. Potential therapeutic approaches targeting 12-LOX would have a great interest in the treatment of mucus overproduction in chronic lung diseases such us CF.
Supported by: Work supported by the French CF association “Vaincre la Mucoviscidose”.

## 80* Proteomic analysis of G551D and R117H-CFTR associated proteins

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Cystic fibrosis (CF) is caused by mutations in the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR). G551D-CFTR, the third most common CF-associated mutation, has been characterized as having a lower open probability than wild-type (WT) channels, and results in a severe clinical phenotype. On the other hand, the missense mutation R117H, which affect ion conductance, is associated with milder disease. Interestingly, CFTR proteins associated with either of these two mutations are located correctly at apical membrane of epithelia, but show different phenotypic severities. Here we describe the development of a proteomic assay to identify proteins, interacting either directly or indirectly with CFTR, whose expressions are variable in G551D and R117H cells, since these variations might be the possible causes of this difference of severity.
With a HeLa cell line transfected with lentivirus and stably expresses CFTR (WT, G551D or R117H), we validated our system using Western blotting and the Cl-sensitive fluorescent indicator 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ), followed by co-immunoprecipitation of CFTR and associated proteins. 2D pattern of immunoprecipitated proteins have been subsequently generated using two dimensional electrophoresis (2-DE). Comparing 2-DE patterns of WT, G551D and R117H, expression-level variations of some protein spots have been observed. Identification of these hotspots by mass spectrometry (MS) is still in progress.
Supported by: Region of Brittany, VLM.